CLAIMS

1. A process for identifying and characterizing mutations leading to a selectable phenotype comprising:

a) generating defined set of overlapping 10 kb PCR products containing random point mutations which encompass the complete chromosome of an organism;

b) transforming pools of 12 PCR products corresponding to 100 kb of the chromosome into a wild-type background;

c) isolating strains of bacteria resistant to compound;

d) re-transforming sensitive bacteria with individual products, 10 kb, from resistant strains thereby identifying a region with one or more mutations;

e) generating smaller PCR products, 1 kb, to further map mutation(s) responsible for phenotype; and

f) sequencing DNA from region conferring resistance to identify the chromosomal mutation.

2. A process for identifying and characterizing quinolone-DNA gyrase interactions using *Neisseria gonorrhoeae* (GC) comprising:

a) mutagenizing randomly the quinolone resistance determining region (QRDR) of *gyrA* using oligonucleotide mediated site-specific mutagenesis with a degenerate oligonucleotide or mutagenizing the entire gene using low-fidelity PCR;

b) transforming these random mutations into a wild-type background;

c) selecting isogenic quinolone resistant mutants following homologous recombination;

d) sequencing the *gyrA* QRDR confirming that Ser91 and Asp95 are independently involved in quinolone inhibition of DNA Gyrase;

e) identifying the following new mutations associated with quinolone resistance in *N. gonorrhoeae*: Asp90 to Glu, Ser91 to Cys, Asp95

to His, Glu161 to Gly, Glu161 to Lys, Asn65 to His, Asp80 to Gly, and Glu62 to Lys; and

f) using these mutants to help to understand the mechanism of action of quinolones, and other type IV topoisomerase inhibitors.

3. Mutations in *Neisseria gonorrhoeae* GyrA associated with quinolone resistance selected from: Asp90 to Glu, Ser91 to Cys, Asp95 to His, Glu161 to Gly, Glu161 to Lys, Asn65 to His, Asp80 to Gly, and Glu62 to Lys.

4. The process according to Claim 1 for identifying and characterizing drug-target interactions.

5. A process for identifying and characterizing a mechanism of action of an antibacterial compound comprising:

generating DNA fragments by polymerase chain reaction amplification of DNA from bacteria under conditions that allow for mutation of the fragments;

allowing one or more of the generated DNA fragments to be incorporated into the chromosome of a bacteria by homologous recombination;

isolating the bacteria that demonstrate resistance to an antibacterial compound; and

identifying the mutation contained in the DNA fragment.

6. A process for identifying mutations contained in the chromosome of a bacteria that results in an identifiable phenotype comprising:

a) generating DNA fragments by polymerase chain reaction amplification of the bacterial chromosome corresponding to regions of the bacterial chromosome which may contain a mutation;

b) allowing one or more of the DNA fragments to be incorporated into the chromosome of a bacteria that does not display the identifiable phenotype by homologous recombination;

c) isolating bacteria that demonstrate the identifiable phenotype;

repeating steps a through c until a single DNA fragment less than about 10 kilobases in length is identified as being responsible for the phenotype; and

identifying the mutation contained in the DNA fragment.

7. The process of claim 5 where the DNA fragments generated collectively encode the entire genome of the bacteria.

8. The process of claim 1 where the bacteria is from the group of genus *Neisseria, Haemophilus, Streptococcus, Staphylococcus,* or *Escherichia.*

9. The process of claim 5 or 6 where the bacteria is from the group of *Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis,* or *Escherichia coli.*

10. The process of claim 5 where the antibacterial compound is a fluoroquinolone.

11. The process of claim 5 where the antibacterial compound is ciprofloxacin.

12. The process of claim 5 where the antibacterial compound is clinafloxacin.

13. The process of claim 5 where the antibacterial compound is dihydroxydiphenylether (DHDPE).

14. The process of claim 5 where the antibacterial compound is Triclosan.

15. The process of claim 6 where the DNA fragments are generated using a bacteria that contain a mutation(s) necessary for resistance to antibacterial compound.

16. The process of claims 1 or 2 in which the antibacterial compound inhibits the growth or survival of the bacteria under any condition.

17. The process of claims 1 or 2 in which the antibacterial compound inhibits the growth or survival of the bacteria in culture.

18. The process of claims 1 or 2 in which the antibacterial compound inhibits the growth or survival of the bacteria in an animal host.

19. The process of claims 1 or 2 in which the antibacterial compound is an inhibitor of type II topoisomerases.

20. The process of claims 1 or 2 in which the antibacterial compound is an inhibitor of FabI.

21. The process of claims 1 or 2 in which the antibacterial compound is an inhibitor of enzymes involved in fatty acid biosynthesis.

22. The process of claim 6 in which a strain of bacteria carrying the mutation was isolated from a culture that had been treated with a chemical mutagen.

23. The process of claim 6 in which a strain of bacteria carrying the mutation was isolated from a culture that had been treated with ultraviolet light.

24. The process of claim 6 in which a strain of bacteria carrying the mutation was isolated from a culture in which the bacteria had been subjected to a mutagenic protocol that consisted of insertion of DNA into the chromosome of the bacteria.

25. Bacteria comprising a protein in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrrhoeae* GyrA and the residue analogous to:
62 is lysine or

63 is arginine or glutamic acid or

65 is histidine or

135 is valine or

161 is glutamic acid or lysine or glycine.

26. *Escherichia coli* strains comprising a GyrA protein in which the amino acid analogous to the *Neisseria gonorrhoeae* GyrA amino acid

62 is lysine or

63 is arginine or glutamic acid or

65 is histidine or

135 is valine or

161 is glutamic acid or lysine or glycine

27. *Neisseria gonorrhoeae* strains comprising a GyrA protein in which the amino acid residue

62 is lysine, or

63 is arginine or glutamic acid, or

65 is histidine, or

80 is alanine or glycine, or

90 is arginine or glutamic acid, or

91 is tyrosine or alanine or cysteine, or

92 is proline, or

95 is arginine or alanine or valine or tyrosine or histidine or glycine, or

114 is histidine, or

135 is valine, or

161 is glutamic acid or lysine or glycine.

28. *Neisseria gonorrhoeae* strains selected from the group consisting of NG-2707, GC318, NG-2721, NG-2711, NG-2706, NG-2717, NG-2687, GC158, NG-2690, GC219, GC291, NG-2691, NG-2720, NG-2723, GC156, NG-2698, NG-2709, NG2716, NG-2719, and NG-2712.

29. A protein comprising in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrrhoeae* GyrA and the residue analogous to:

62 is lysine or

63 is arginine or glutamic acid or

65 is histidine or

135 is valine or

161 is glutamic acid or lysine or glycine.

30. *Neisseria gonorrheae* GyrA protein comprising amino acid substitutions when residue

62 is lysine, or

63 is arginine or glutamic acid, or

65 is histidine, or

80 is alanine or glycine, or

90 is arginine or glutamic acid, or

91 is tyrosine or alanine or cysteine, or

92 is proline, or

95 is arginine or alanine or valine or tyrosine or histidine or glycine, or

114 is histidine, or

135 is valine, or

161 is glutamic acid or lysine or glycine.

31. Bacteria comprising a protein that is at least 30% identical to the sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid residue corresponding to

15 is valine, or

20 is threonine, or

23 is glycine, or

25 is valine, or

51 is threonine, or

91 is threonine, or

93 is cysteine or serine, or

95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

32. A Neisseria gonorrhoeae strain selected from the group consisting of NG-2669, NG-2654, NG-2651, NG-2670, NG-2660, NG-2641, NG-2639, NG-2638, NG-2640, NG-2648, NG-2657, NG-2656, NG-2653, NG-2658, NG-2663, NG-2642, NG-2671, NG-2652, NG-2661, NG-2644, NG-2667, NG-2665, NG-2655, NG-2643, NG-2666, NG-2664, NG-2647, NG-2646, NG-2650, NG-2649, NG-2645, NG-2659, NG-2662, and NG-2672.

33. An *Escherichia coli* strain comprising a FabI protein with the amino acids analagous to the ones described in claim 31 with the exception of mutations resulting in changing residue
93 to alanine or serine or cysteine or valine,
159 to threonine or,
203 to leucine.

34. A protein comprising at least 30% identical to the sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid residue corresponding to
15 is valine, or
20 is threonine, or

23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

35. A *Neisseria gonorrhoeae* FabI protein comprising the amino acid corresponding to residue:
15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or

147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

36. The process of screening compounds for antibacterial activity comprising:
generating DNA fragments by polymerase chain reaction amplification of DNA from an entire genome of a bacteria under conditions that allow for mutation of the fragments;
allowing one or more of the generated DNA fragments to be incorporated into the chromosome of a bacteria by homologous recombination;
isolating the bacteria that demonstrate resistance to an antibacterial compound;
identifying the mutation contained in the DNA fragment;
contacting the bacteria with compounds; and
evaluating the compounds for antibacterial activity.